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Determinants of the Fynbos/Succulent Karoo biome boundary: Insights from a reciprocal transplant experiment

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ABSTRACT

Boundaries between Fynbos and Succulent Karoo vegetation in the Greater Cape Floristic Region are frequently characterised by sharp transitions from sandy, dystrophic to loamy, mesotrophic soils, together with a more gradual climate transition from cooler, wetter conditions typical of Fynbos at higher elevations to warmer, drier conditions at lower elevations typical of Succulent Karoo. There is very high species turnover across these boundaries, providing an opportunity to disentangle the relative roles of climate and soil type in determining the biome boundary. A fully reciprocal transplant approach was employed here to investigate this question, using three species from each biome occurring naturally in close proximity at Jonaskop, Western Cape. Greenhouse-germinated and established seedlings of all species were planted into both sandy, dystrophic and loamy, mesotrophic soils typical of each biome at four sites along an elevational transect (elevations 545 m, 744 m, 953 m, 1303 m) at Jonaskop, and their growth and survivorship monitored for 7 months.

At least one site on the elevation gradient represented annual climate rainfall and temperature conditions during the experimental period typical of the range edge for each of the selected species, this typically being the lowest elevation site (warm × dry) for Fynbos species (*Protea humiflora*, *P. magnifica* and *P. amplexicaulis*), and the highest elevation site (cool × wet) for Succulent Karoo species (*Ruschia lineolata*, *Drosanthemum speciosum* and *Pteronia incana*). Fynbos species showed significant adverse responses to loamy mesotrophic soil, with highly significant reductions in growth and high and rapid rates of mortality relative to their native soil. Fynbos species showed somewhat reduced growth and survival at the lowest elevation when grown in native soils, but demonstrated significant interaction between soil type and elevation with much lower growth and survival at the lowest elevation on the loamy soils. Surprisingly, all the Fynbos species showed reductions in growth and survival at the highest elevation, with significant reductions in two of the three species. Succulent Karoo species, by contrast, showed very few significant performance differences between soil types and few significant soil × elevation interactive effects, but did show significant growth and survival responses to elevation, with high growth and survival at mid-level elevations, far higher than their natural extent at the site.

These results suggest that the selected Succulent Karoo species are neither edaphically nor climatically constrained from establishing and growing in sandy dystrophic soils and cool climates typical of the Fynbos vegetation along this elevation gradient, but that Fynbos species are strongly limited both edaphically and climatically from growing under conditions typical of the Succulent Karoo. We propose that Succulent Karoo elements may be excluded competitively or through disturbance from colonising sandy dystrophic soils at higher elevations in Fynbos vegetation, with fire regime most likely responsible for maintaining the sharply delineated boundaries between these biomes. This is because fire would strongly exclude non-fire adapted Succulent Karoo species at and above the biome boundary, while loamy soils and climate together would strongly exclude Fynbos species from the heavier soils of the Succulent Karoo. The relative climate and soil affinities of these biomes, accentuated by the role of fire, could therefore provide a coherent explanation for biome boundaries in the Greater Cape Floristic Region. We note however that the limited species selection in this study precludes a conclusive general result, and that several interesting questions remain about soil, climate and disturbance determinants across this biome boundary.

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1. Introduction

Both Fynbos and Succulent Karoo Biomes are renowned for their exceptional plant species diversity (Goldblatt, 1997; Cowling et al., 1999). However, apart from sharing similarities in climate seasonality and the virtual absence of C_4 grasses, these biomes are distinct in most other biophysical, ecological and phylogeographic respects (Mucina et al., 2006; Rebelo et al., 2006). The boundary between these biomes, most often characterised by a discontinuous transition from sandy, dystrophic to loamy, mesotrophic soils, and a more continuous climate transition from cooler wetter to warmer drier conditions, is therefore biologically and ecologically interesting. A large amount of literature exists on the determinants of southern African biomes, as well as on the boundaries of the Fynbos Biome in particular (Cowling and Holmes, 1992; Rutherford and Westfall, 1994; Rutherford, 1997; Lechmere-Oertel and Cowling, 2001; Bond et al., 2003). The observed high species turnover across Fynbos-Succulent Karoo boundaries has variously been ascribed to temperature and rainfall variation, associated with aspect and elevation, as well as different soil conditions (Lechmere-Oertel and Cowling, 2001), and the result of differences in fire regime (Oliver et al., 1983; Cowling and Holmes, 1992; Goldblatt, 1997). Geology and soil type have widely been invoked as determinants of the biome boundary (Cowling and Holmes, 1992; Cowling et al., 1997; Milton et al., 1997; Lechmere-Oertel and Cowling, 2001). However, the factors that determine this boundary have rarely been explored using field reciprocal transplant experiments.

Fynbos and Succulent Karoo biomes make up the majority of the Greater Cape Floristic Region (Bergh et al., 2014) which is characterised bioclimatically by winter rainfall and summer drought, except at high elevations where summer precipitation may match that received in winter (Agenbag et al., 2008), and towards its eastern reaches. The Succulent Karoo is associated with drier and warmer conditions, and is found on plains and lower slopes with annual rainfall between 20 and 300 mm and summer temperatures reaching up to 44 °C during summer, while Fynbos is found on sandy lowland coastal plains as well as mountains, but not in areas where annual rainfall is below 200 mm, and less comonly on shale-derived loamy mesotrophic substrates (Low and Rebelo, 1996; Mucina et al., 2006; Rebelo et al., 2006). Shale derived loamy mesotrophic soils have distinct soil water holding and release characteristics from sand derived soils more typical of Fynbos, potentially providing an explanation for the lack of success of fynbos elements on shales. Indeed, at higher elevations – with cooler air temperature and higher rainfall – distinct fynbos vegetation types can be found on heavier soils typical of shale bands (Mucina and Rutherford, 2006), suggesting that high enough rainfall could overcome the water limitations imposed by shale soils on fynbos elements under drier conditions.

Apart from the water relations differences between these soil types due to the effects of higher clay content, soil differences could also be responsible for nutrient limitations and/or toxicity effects. However, this seems to be less likely as a potential biome boundary determinant, as the Cape Floristic Region is characterized by a diversity of geological formations and soil types (Bradshaw and Cowling, 2014), and Fynbos is found on a variety of soil types, ranging from derivatives of ancient, highly leached sandstone formations in the mountains, to moderately fertile finer-grained soils derived from shales and granite (mostly on the lowlands) and complex sequences of nutrient poor acidic and alkaline sands of Aeolian and marine origin along the coast (Cowling et al., 1997). Most of the underlying geological formations of the Fynbos Biome extend beyond the biome borders (Partridge, 1997), and adjacent biomes share many of the sandy soil types. Therefore, at the biome level, underlying geology seems to have an inconsistent level of importance in delimiting biome boundaries, possibly due to interactions with climate.

The role of soil type *per se* has been explored experimentally at both biome and community levels. In a controlled greenhouse experiment specifically concerned with the Fynbos-Succulent Karoo boundary, Lechmere-Oertel and Cowling (2001) found that a combination of soil type and moisture levels controlled seedling growth and survival. Fynbos seedlings survived on sandstone as well as shale derived soils under mesic conditions, but died rapidly under xeric conditions in both soil types. Succulent Karoo seedlings survived well in both soil types and under both moisture regimes. Euston-Brown (1995) performed one of the very few reciprocal transplants of this type, encompassing eight vegetation types in the Kouga Mountains, Eastern Cape, and found that Fynbos plants survived well at all sites except in the most arid areas, which were associated with shale derived soils. Succulents grew well at all sites except at the highest elevations.

At the level of plant community, many species assemblages are associated with specific soil types (Low and Rebelo, 1996). For example Richards et al. (1997a) found that soil type, rather than competition, explains replacement of Proteaceae species pairs on a sandstone-limestone edaphic gradient, and further showed how community boundaries are associated with variations in soil nutrient levels (Richards et al., 1997b). Mustart and Cowling (1993a), however, in a reciprocal transplant experiment found that soil type did not limit the germination and growth of seedlings and did not explain the distribution of edaphically restricted Proteaceae species on the Agulhas Plain. Latimer (2006) and Latimer et al. (2009) used reciprocal transplant experiments to show that species turnover across large scale climatic gradients in high-elevation white Proteas was determined less by abiotic and competitive effects, but more by dispersal limitation, and that these species both grew well and were projected to maintain positive population growth rates well outside of the bioclimatic limits suggested by their observed geographic ranges.

Apart from climate, geology and soil, disturbance is considered one of the main sculptors of vegetation patterns in southern Africa (Bond, 1997; Bond et al., 2003). In the Fynbos Biome, fire drives ecological processes such as regeneration, succession and vegetation dynamics, and because tolerance of fire requires adaptive responses in plants (Bond and Van Wilgen, 1996; Keeley et al., 2012), fire would be a critical factor in preventing species from other biomes from colonizing Fynbos. Succulent Karoo species are intolerant of fire, but it has been found that in the absence of fire, thicket and succulents have successfully invaded Fynbos (Cowling and Pierce, 1988). Wildfire is also thought to play a critical role in differentiating the biomes, but is likely an emergent property of vegetation type, due both to fuel accumulation and flammability traits. Wildfire is generally limited to Fynbos vegetation, and rarely burns into Succulent Karoo (though Renosterveld vegetation does burn on heavier soils under arid conditions, and the dominant renosterbos, *Elytropappus rhinocerotis*, has long been recognized as favouring the spread of fire (Levyns, 1929a,b,c, 1935, 1956)). A few exceptions of wildfire occurring in the Succulent Karoo have been recorded, but had devastating impacts especially on succulent components of the flora (Forrester, 1988; Rahlao et al., 2009).

Climate, soil type and wildfire need to be disentangled to develop a full and general explanatory understanding of the determinants of boundaries between Fynbos and Succulent Karoo. This would be especially useful to project how this boundary might respond, if at all, to climate change, both in the past and into the future. Bioclimatic modeling of biome distributions under future climate scenarios indicate a contraction of Fynbos and a southward shift of Succulent Karoo into areas currently occupied by Fynbos (Hannah et al., 2002; Midgley et al., 2003). These models however consider only climatic limitations to biome distributions, and ignore other biotic and abiotic factors involved in shaping biome boundaries.

The boundary between the Fynbos and Succulent Karoo biomes, with high rates of species and growth form turnover, occurs over short distances (sometimes as little as a few tens to hundreds of metres). This is especially evident on dry, equator-facing slopes of the Cape Floristic Region (Cowling and Holmes, 1992). This natural situation lends itself to a reciprocal transplant experimental approach to

tease apart climate and soil determinants of biome boundaries directly, thus allowing the role for fire determinants to be inferred. Reciprocal transplant experiments are extremely useful in disentangling various biotic and abiotic determinants of community boundaries (Ewanchuk and Bertness, 2004; Pennings et al., 2005). Such experiments are also very valuable in testing climate change predictions (Newton et al., 2001). Transplanting species across elevational gradients allows the simulation of future climate change scenarios (Bruelheide, 2003; Link et al., 2003).

We explored how representative elements of Fynbos and Succulent Karoo established, grew and survived over the critical seedling establishment phase in both soil types and across an elevation-associated climate gradient in a fully reciprocal transplant experiment. In our study system, all vegetation elements were exposed, under field conditions, to both soil types under a range of optimal and sub-optimal rainfall and temperature conditions.

2. Materials and methods

2.1. Study system

The study system is an elevational transect providing both temperature and moisture gradients. It is situated on the north-facing slope of Jonaskop in the Riviersonderend Mountains, within the Mediterranean climate region of the Western Cape (Agenbag et al., 2008; see Supplementary materials, Fig. A). Succulent Karoo vegetation is found on loamy, mesotrophic shale derived soils on the lower warmer and drier end of the transect (up to 600 m elevation). Mountain Fynbos is found on sandstone derived soils between the mountain summit (1694 m) and mid elevations (±800 m). Vegetation types are named according to Low and Rebelo (1996).

Weather stations placed at selected points along the transect, which spans a change of roughly 1000 m in elevation, indicate that temperatures decrease on average by 0.38 °C with every 100 m rise in elevation. The lowest weather station recorded, roughly half the mean annual rainfall of the weather station located near the mountain summit. Data for total monthly rainfall, mean, maximum and minimum temperatures recorded during the experiment at the top (site A), upper middle (site B) and lower end (site D) of the transect are summarized in Fig. 1.

Sandstone and shale derived soils sampled at selected elevations on the transect, and used as the growing medium in this experiment, differed strongly in phosphorous and magnesium content but not in terms of other nutrients (N, Ca, K and Na) and pH (Agenbag et al., 2008).

2.2. Study species

Seeds were collected on the transect of several species dominant within each of the Fynbos and Succulent Karoo, however, only six species germinated in sufficient numbers to be used in the transplant experiment. These included three Fynbos species, *Protea magnifica* Link (Proteaceae), *Protea amplexicaulis* (Salisb.) R. Br. (Proteaceae), *Protea humiflora* Andrews (Proteaceae), and three Succulent Karoo species, *Drosanthemum speciosum* (Haw.) Schwantes (Mesembryanthemaceae), *Ruschia lineolata* (Haw.) Schwantes (Mesembryanthemaceae) and *Pteronia incana* (Burm.) DC. (Asteraceae) (see Supplementary materials for more detail; Figs. B & C). All seeds were treated with commercially available smoke primer, since it has been shown that fynbos species (Brown and Botha, 2004) and many succulents have improved germination in response to smoke treatment (Pierce et al., 1995). Larger seeds (Proteaceae) were germinated first on moistened filter paper in temperature and light controlled growth chambers according to prescriptions by Mustart and Cowling (1991) before being planted into seedling trays. Smaller seeds were sown directly into seedling trays containing either sandstone or shale derived soils collected on the transect. All seeds were germinated during April 2004.

Seedlings were kept under greenhouse conditions for roughly two months after germination, and watered daily. About three weeks before planting into the field, seedlings were moved into direct sunlight and watering was gradually reduced to twice a week to harden them, after which they were planted into the field site during late July 2004.

2.3. Transplant design and statistical analysis

Field sites were selected at four selected elevations: near the top of the mountain in Fynbos (1303 m; site A); upper middle elevation in Fynbos (953 m; site B); lower middle elevation in Fynbos (744 m; site C); and low elevation in Succulent Karoo vegetation (545 m; site D). These sites were selected because vegetation analysis (Agenbag et al., 2008) and a phenological study (Agenbag, 2006) suggested that the climate shifts associated with these elevation differences had notable effects on shoot growth timing and extent in Fynbos and Succulent Karoo species, and thus into climatic conditions controlling growth and distributions of species along the mountain slope. A simple block design was used, where each of the four sites consisted of 14 containers, each 0.6 m wide, 0.64 m long and 0.3 m deep. Containers were placed into a randomized 7 × 2 block on top of the soil surface. Seven containers were filled with sandstone-derived soil collected from site B

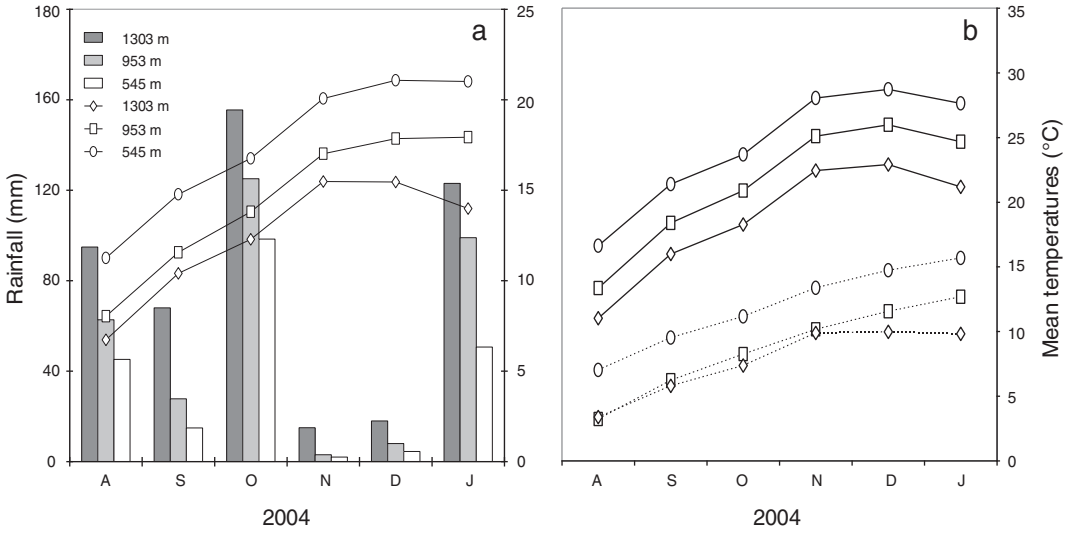

Fig. 1. Temperature and rainfall recorded at selected elevations during the experiment. (a) Total monthly rainfall (bars) and mean monthly temperature (lines). (b) Mean daily maximum temperatures (solid lines) and mean daily minimum temperatures (dashed lines).

and another 7 filled with shale-derived soil collected from site D. The bottoms of the containers were partially isolated from the soil below with a layer of coarse gravel and plastic sheeting with regularly spaced holes to facilitate drainage. An outer wooden frame in-filled with sand to a width of a few centimetres and to soil level in the containers provided thermal insulation around the outer perimeter of the 7 × 2 block of side-by-side containers (see Supplementary materials, Fig. A).

Depending on germination success, either two (*Protea amplexicaulis*), three (*Protea magnifica*, *Pteronia incana*) or four (*Protea humiflora*, *Drosanthemum speciosum*, *Ruschia lineolata*) seedlings per species were planted into randomly assigned positions in each container. Seedlings were watered after transplanting and again two days later, but were subsequently left to grow under natural climate conditions.

The experiment ran from July 2004 to end January 2005. Seedling sizes were measured monthly for roughly eight months (height and canopy diameter measurements were taken) to include a winter (wet) as well as summer (dry) period. Seedling sizes were calculated by multiplication of height × diameter. At the end of the experiment, total seedling growth was calculated as the difference in seedling size between the natural log of the largest measurement taken, and that of the initial measurement taken on the day the seedlings were transplanted. This obviated the need to exclude data from seedlings that had died. Mean total growth for each container was used in a main effects ANOVA to test for the interactive effects of categorical factors climate (represented by sites A to D) and soil type on seedling growth. The proportion of seedlings that had died by the end of the experimental period was arcsin transformed and used in a main effects ANOVA as done for the growth data (R core development team, 2013).

The relative climatic suitability of each of the four sites was assessed using a rudimentary bioclimatic analysis of species observed distributions via a method similar to Westman (1991) (see Supplementary materials, Figs. B and C).

3. Results

The assessment of relative climatic suitability for the four transplant sites revealed that they represented both optimal and sub-optimal rainfall and/or temperature growing conditions for all six species (see Supplementary materials). At least one site on the elevation gradient represented annual climate rainfall and temperature conditions during the experimental period typical of the local range edge for each of the selected species, this typically being the lowest elevation site (warm × dry) for Fynbos species (*Protea humiflora*, *P. magnifica* and *P. amplexicaulis*), and the highest elevation site (cool × wet) for Succulent Karoo species (*Ruschia lineolata*, *Drosanthemum speciosum* and *Pteronia incana*) (see Supplementary materials).

Initially, *Protea* seedlings tended to grow fastest at the warmest and driest low elevation site (D, 545 m) on their native soil (data not shown), but when the dry summer season commenced, growth rates declined greatly at this site, while growth rates of seedlings at all of the higher elevation sites increased rapidly. By the end of the experiment all *Protea* seedlings tended to be largest at the lower mid-elevation site C (744 m) (Fig. 2). *Protea* seedlings grew rapidly in their native soils from the beginning of the experiment, but remained much smaller, or died, in the loamy soils throughout the experiment (Figs. 2, 3).

By contrast with Fynbos species, no soil effects were found in the growth of *Drosanthemum speciosum* and *Ruschia lineolata* seedlings, but *Pteronia incana* performed better in native loamy soil, a trend that became apparent during summer (data not shown). Similar to the patterns found in Fynbos seedlings grown at higher elevations, Succulent Karoo seedlings grew little during winter, but growth rates increased rapidly as temperatures rose during spring months. As was the case with the Fynbos species, Succulent Karoo species grew larger at the three lower elevation sites, but in both soil types (Fig. 2).

Climate had a significant effect on seedling growth in all species (Table 1), while soil type had a significant effect on seedling growth for all three *Protea* species, as well as *Pteronia incana*, but not for the Mesembryanthemaceae species (Table 1). In all cases of significant soil effects, Fynbos seedlings performed best on their native sandy soils. Only two species showed significant climate × soil interaction for growth, with *Protea amplexicaulis* showing sharply higher growth at mid- and high elevations in native sandy soil, and *P. magnifica* showing declining growth at higher elevations in non-native loamy soil (Table 2).

Climate had a significant effect on seedling survival in all species, while soil type affected only Fynbos species survival significantly, with no soil effects on Succulent Karoo seedling survival (Table 2). *Protea* seedlings that died in loamy soils did so within two months of transplanting at all elevations (data not shown), but generally survived well in native sandy soils, with only a few mortalities recorded at the end of the dry summer period at the lower elevation sites C and D. *Protea amplexicaulis* and *P. humiflora* seedlings also showed high rates of mortality within two months of transplanting (data not shown) in native sandy soil at the highest elevation site A. The Succulent Karoo species' seedlings survived relatively poorly in both soil types at the highest elevation site A, but otherwise no clear elevational pattern or soil preference was apparent, with relatively high survival at both the upper mid-elevation site B and lower elevation site D for all three species (Fig. 3). *Pteronia incana* and *D. speciosum* had poorest survival in loamy soil at the highest elevation site A.

Only two species shows significant climate × soil interaction for survival, with *Protea amplexicaulis* showing higher survival at lower elevations in native sandy soil, and *P. magnifica* showing higher survival at higher elevations in native sandy soil (Table 2).

4. Discussion

Despite a limited number of species investigated, and given the limitations due to phylogenetic bias of the final experimental design, we provide evidence to suggest that an interaction between drivers is at play in determining the distribution of species along the Fynbos-Succulent Karoo biome boundary. We first discuss controls at the interface between these two biomes, and then explore implications under a future warmer and drier climate, including the question of whether Succulent Karoo species could invade Fynbos if the climate were to change in this way.

4.1. What controls the Fynbos-Succulent Karoo boundary?

Climate significantly controlled the growth and survival of the seedlings of all species tested, and in general they all revealed highest growth and survival at mid-elevation sites. The cool temperatures of the highest elevation site appeared to have a significant adverse effect on the growth and survival of all species except *Protea magnifica*. This pattern of growth and survival with elevation concurred relatively well with expectations for Fynbos species based on their geographical distribution, and their natural distribution on the transect., but the magnitude of the drop in growth and survival at the highest elevation site was somewhat unexpected, given the naturally higher elevation range than the Succulent Karoo species. By contrast, there was much less concurrence between the expected growth and survival of Succulent Karoo species and their natural distribution on the transect, as they showed significantly elevated growth rates and survivorship under the cooler and wetter conditions of the mid-elevation elevation sites, regardless of soil type. Succulent Karoo species did tend to show highest survival at the lowest elevation sites as expected, where growth rates were also generally higher.

Soil type emerged as a strong controlling factor for Fynbos species, but not for the Succulent Karoo species we studied. The rapid adverse response of Fynbos species to transplantation into non-native loamy

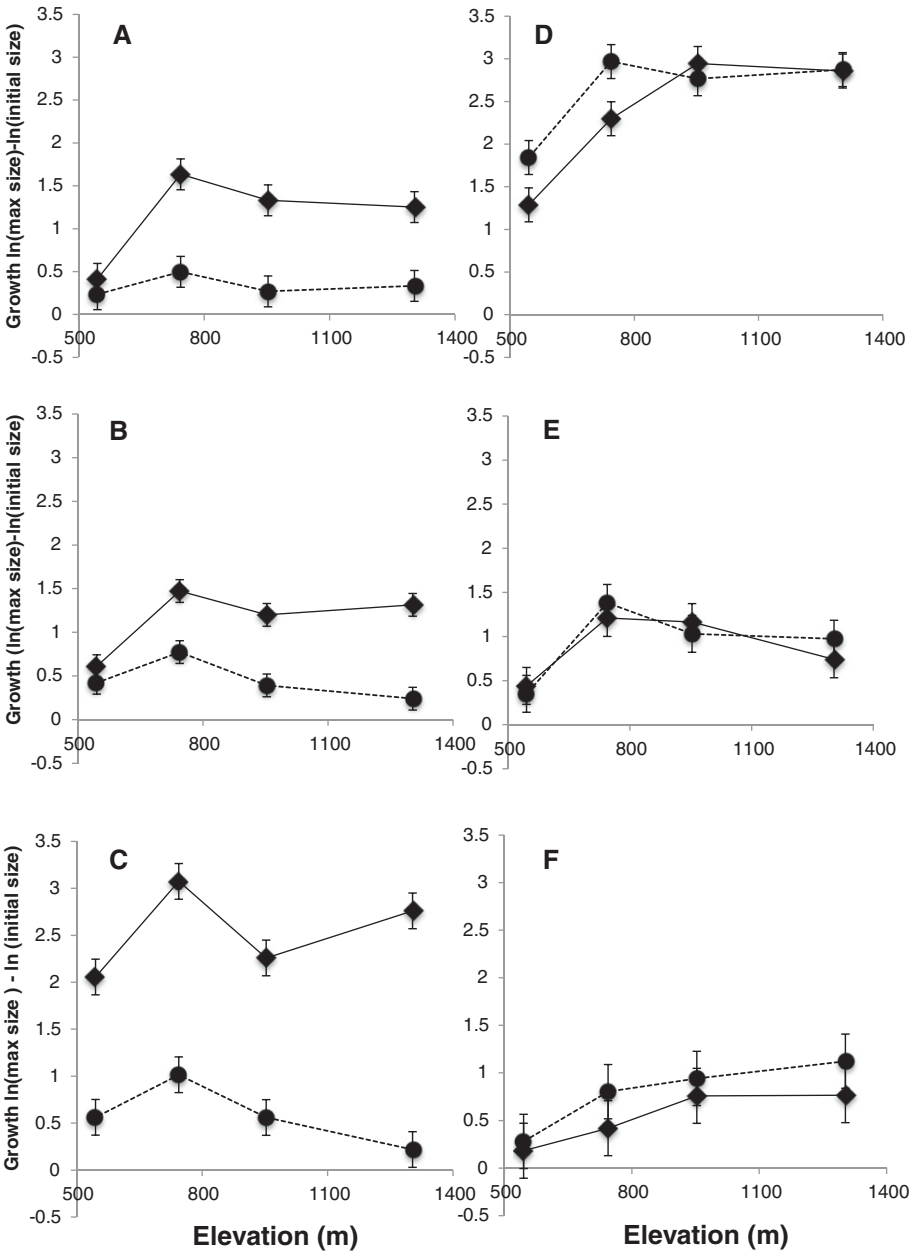

Fig. 2. Effects of elevation (associated with different temperature and rainfall at which experimental plots were placed) and soil type (Fynbos soil = diamonds; Karoo = circles) on the growth of fynbos seedlings A) *Protea amplexicaulis*, B) P. *humiflora*, C) *P. magnifica* and karoo seedlings D) *Rushia lineolata*, E) *Drosanthemum speciosum*, F) *Pteronia incana*.

soil indicates a toxicity effect. Studies on root physiology of Australian Proteaceae, which are also found on highly leached, extreme nutrient poor soils indicate that Proteaceae species suffer from phosphorous toxicity when growing in soils containing only marginally higher levels of P due to an inability of the roots to control P uptake (Shane et al., 2004). Studies indicate that Cape Proteaceae are similarly sensitive to increases in soil P levels (Hawkins et al., 2009; Cramer et al., 2014), possibly explaining why the *Protea* species in this study did not grow and survive well on relatively more nutrient rich shale derived soil. However, the interactive effects of soil and climate found for *Protea amplexicaulis* and *P. magnifica* indicate that these species could grow and survive relatively better on shale derived soils under moister conditions. This is consistent with the findings of Lechmere-Oertel and Cowling's (2001) greenhouse study on Proteaceae. It appears then, that a combination of low rainfall (water deficit) and heavy, nutrient rich soils restrict Fynbos species from shifting into the more arid loamy soils of the Succulent Karoo.

The fact that neither soil type nor climate is limiting the two Mesembryanthemaceae species from intruding into Fynbos raises the question why are they not in Fynbos? Fire is an obvious factor to consider – these two species are possibly not fire adapted. However, life histories of other Mesembryanthemaceae species such as *Erepsia* which are associated with Fynbos could possibly hint at an additional factor. *Erepsia* species are often highly abundant in early post-fire regeneration stages of Fynbos but disappear as the vegetation matures and becomes denser and taller. Fynbos species are very likely superior competitors over Mesembryanthemaceae and possibly other Succulent Karoo species as well, marginalizing their realized niches into more stressful arid environments. Such patterns of low competitive ability associated with wider tolerance of environmental conditions have been found for other species (Parmesan et al., 2005; Veldkornet et al., 2015–in this issue). We speculate that under higher rainfall, fire likely excludes Succulent

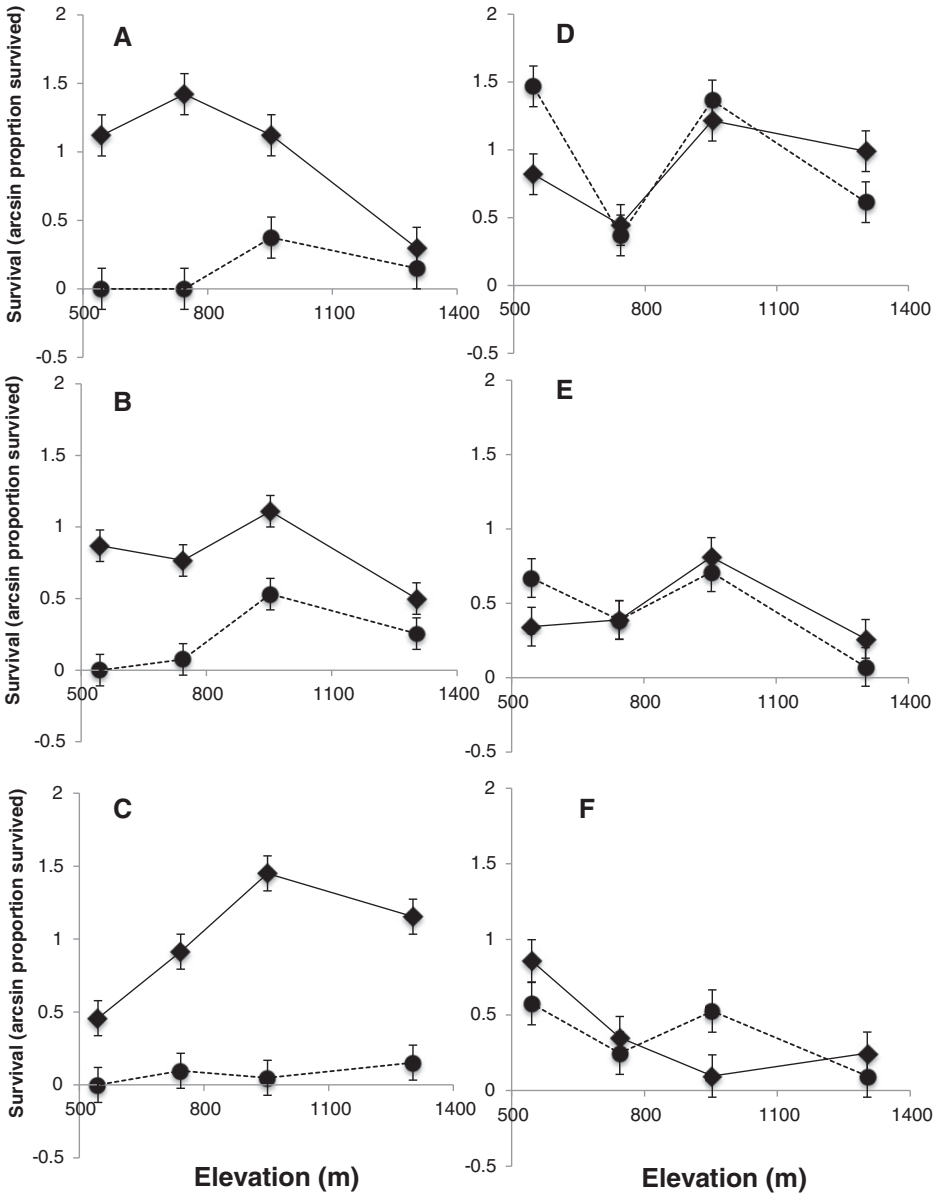

Fig. 3. The effects of elevation (associated with different temperature and rainfall at which experimental plots were placed) and soil type (Fynbos soil = diamonds; Karoo = circles) on the survival of fynbos seedlings A) *Protea amplexicaulis*, B) *P. humiflora*, C) *P. magnifica* and karoo seedlings D) *Rushia lineolata*, E) *Drosanthemum speciosum*, F) *Pteronia incana*.

Karoo even from shale derived soils because of the presence of fire tolerant Renosterveld species that often interject in a band between Fynbos and Succulent Karoo. Again, where rainfall drops low enough to hinder biomass accumulation, it is possible that this band of vegetation would disappear, and Fynbos and Succulent Karoo species would intermingle.

Table 1
Main effects ANOVA for the effects of elevation (associated with different temperature and rainfall at which experimental plots were placed) and soil type on the growth of Fynbos and Karoo seedlings. Note residual degrees of freedom in all cases = 48.

Species	Elevation df = 3		Soil df = 1		Interaction df = 3	
	F	p	F	p	F	p
Fynbos						
Protea humiflora	7.246	<0.001	56.535	<0.001	4.034	0.012
Protea amplexicaulis	5.825	0.002	41.797	<0.001	2.970	0.041
Protea magnifica	6.169	0.001	216.166	<0.001	3.021	0.039
Karoo						
Pteronia incana	5.961	<0.001	15.446	<0.001	2.344	0.085
Drosanthemum speciosum	6.891	<0.001	0.094	0.761	0.388	0.762
Ruschia lineolata	19.235	<0.001	3.523	0.067	2.122	0.110

Table 2
Main effects ANOVA for the effects of elevation (associated with different temperature and rainfall at which experimental plots were placed) and soil type on the survival of Fynbos and Karoo seedlings. Note residual degrees of freedom in all cases = 48.

Species	Elevation df = 3		Soil df = 1		Interaction df = 3	
	F	p	F	p	F	p
Fynbos						
Protea humiflora	6.345	0.001	52.837	<0.001	2.565	0.066
Protea amplexicaulis	5.031	0.004	65.443	<0.001	6.639	<0.001
Protea magnifica	7.101	<0.001	116.668	<0.001	5.317	0.003
Karoo						
Pteronia incana	5.600	0.002	0.067	0.797	2.475	0.073
Drosanthemum speciosum	6.899	<0.001	0.008	0.930	1.434	0.245
Ruschia lineolata	13.684	<0.001	0.653	0.423	4.141	0.011

4.2. How might climate change affect Fynbos species?

Site D was considered representative of possible future climate conditions for the upper elevation sites with warmer winter conditions that initially caused seedlings of all three *Protea* species to grow much faster and larger than any other site along the transect. This suggests that higher winter temperatures predicted by climate change might actually enhance species' growth. Drier conditions however impaired growth to such an extent that seedlings at moister but cooler sites (A and B) outgrew seedlings at site D towards the end of summer. Only 6.4 mm rain was recorded at the Karoo site (D) from November to December 2004; the effect of this was evident on seedling growth as well as survival, with many *P. magnifica* and *P. humiflora* seedlings dying during January 2005. However only *P. magnifica* had higher seedling mortality at site D than anywhere else on the transect, suggesting that mortality might not be such a serious concern and that these three *Protea* species might actually be quite resilient to warmer and drier conditions. This is confirmed in studies by Midgley (1988) and West et al. (2012) who found that seedlings, juveniles and adults of several other Proteaceae species are remarkably resilient to summer drought. In contrast, Lechmere-Oertel and Cowling (2001), found *Leucadendron* seedlings to be directly limited by water deficit while Mustart and Cowling (1993a, 1993b) came to similar conclusions when conducting field and laboratory experiments on survival of newly emerged proteoid seedlings. Overall, this points to considerable variation in drought response in Fynbos species (and at different demographic stages), as confirmed by ecophysiological measurements along the Jonaskop transect that included adult Proteaceae and other Fynbos species (Jacobsen et al., 2007, 2009).

A point to consider is that seedlings in this experiment were not established under drought conditions. Midgley (1988) found slightly, but not significantly higher mortalities in seedlings that were less than four months old at the start of summer due to fires occurring during winter rather than autumn. Drought sensitivity in *Protea* seedlings is likely more apparent in the very early stages (Mustart et al., 2012), and once the seedlings have become established they may be able to tolerate variable levels of drought stress. Furthermore neither this study nor that of Midgley (1988) assessed the effects of prolonged drought on Fynbos seedling survival. Had site D not received relatively high rainfall (50.6 mm) during January 2005, survival patterns recorded in this experiment might have shown many more seedling deaths recorded at the last measurement at the end of January 2005. Finally, higher initial growth at the warmer sites may well have improved survival into the dry season for these individuals.

4.3. Will Succulent Karoo invade Fynbos?

Palaeo-ecological studies concerning vegetation responses to past changes in climate have indicated that changes happen most rapidly in marginal zones, along biome boundaries, while vegetation tends to persist in the most favourable habitats for a very long time after the climate change has taken place (Cole, 1985; Peteet, 2000; Bergh et al., 2007). Dry north-facing slopes within the Fynbos Biome, especially in areas where Fynbos intersects directly with Succulent Karoo are therefore probably most at risk of being replaced by Succulent Karoo, while Fynbos in other, higher rainfall areas such as south-facing mountain slopes will probably be able to persist as a vegetation type in spite of the effects of climate change.

It is tempting to speculate about the role of interjecting Renosterveld species under climate change, as these speculations may provide a fruitful future avenue for research. We know that under lower temperatures and higher rainfall, Renosterveld is spread widely onto shale soils (Rebelo et al., 2006). It is likely, however, that grasses (which can be regarded as the Fynbos restiod analog in current Renosterveld), would not have accompanied these shifts during the colder, wetter Pleistocene as they are heavily dependent on growing degree days (Woodward, 1987). However, with predicted future rising temperatures, and an increasing summer/spring rainfall component (Altwegg et al., 2014), we suggest that there is potential for a new, grass-dominated (possibly alien), fire-prone shrubland to emerge, much like that seen in southern California (Keeley et al., 2012). With higher temperatures and even lower rainfall, interfacing Renosterveld vegetation may even collapse completely; we speculate that under these conditions Succulent Karoo could gradually move up-slope, but only in the absence of fire. Obvious next experiments should involve an investigation of the fate of Renosterveld species such as *Elytropappus* and grasses in this or a similar Cape system.

Nonetheless, with some Fynbos species being very long-lived (they can resprout after fire), and should further studies confirm their competitive superiority over Succulent Karoo, Fynbos is likely to display considerable biological inertia (Von Holle et al., 2003) against invasion by Succulent Karoo even in marginal habitats. Von Holle et al. (2003) suggest that competitively inferior but environmentally more tolerant invaders require openings in the more competitive resident vegetation before they are able to establish. In the Fynbos context, fires are likely to provide such openings: should a fire be followed by a prolonged and severe drought, Fynbos species could fail to re-establish and such areas could be opened up for invasion by adjacent more drought tolerant Succulent Karoo species.

5. Conclusion

The work presented here made use of an elevational transect incorporating a transition from heavier shale derived soils to sandstone derived soils, overlaid by a climatic gradient of cooling temperatures and increasing rainfall with increasing elevation, in order to explore the relative role of soil type and climate in determining the boundary between two distinct biomes that are dominant in this winter rainfall region. The reciprocal transplant approach allowed soil types to be distributed across the entire climatic gradient, providing insights into the seedling survivorship and growth of representative elements of both the Succulent Karoo and Fynbos Biomes. The use of this approach exposed the selected species to optimal and sub-optimal rainfall and temperature conditions as determined by analysis of their observed natural distributions, on both soil types. Overall, the growth of Fynbos species was more strongly affected by soil type, while that of Succulent Karoo species was more strongly affected by climate. Results for seedling survivorship matched this result, with the particularly significant rapid mortality of Fynbos species on the non-native shale-derived loam soils under all climate conditions. This suggests that this biome boundary is not the result of a simple set of species edaphic and climatic controls leading to the turnover and species replacement across the boundary, and demands a more complex explanation that may have relevance for understanding changes under past climate change. These findings also raise the question of the position of the Renosterveld vegetation type that is often interjected between these two biomes, and has structural and functional similarities to both, and we will elaborate on this point below.

With respect to climate control, it was also noteworthy that, although climate had a significant effect on growth and survival of seedlings of all species, these responses were not fully consistent with the inferred optimal climatic range derived from their local natural distributions, with the seedlings of some species from both biomes surviving and growing well outside of their elevational range. Succulent Karoo species particularly, while showing their highest growth under the expected climatic conditions, only showed a significant drop in growth at much cooler temperatures at the highest point in the transect, which was well outside of their natural elevational range. Fynbos species, by contrast, showed equal or higher growth rates under the higher temperatures and lower rainfall conditions at lower elevations than would be expected based on their natural distributions. This finding is particularly relevant for understanding the determinants of the biome boundary when considered in conjunction with findings for soil type effects.

Soil type strongly limited Fynbos seedling survival and growth, but not that of Succulent Karoo seedlings. When considered in conjunction with the climate controls discussed above, this suggests that Fynbos species are simply excluded from the heaver soils under the warmer conditions of the lowest elevation, either due to toxicity or soil water relations effects. The reciprocal explanation cannot hold for Succulent Karoo species, which showed no sign of either soil or climate limitation under conditions typical at higher elevations. We suggest therefore that competition and fire, rather than climate and soil type, likely prevent the Succulent Karoo species from intruding into Fynbos, while climate and soil type are strong controllers of the distribution of the Fynbos species.

This explanation provides a window on understanding of the situation of Renosterveld vegetation. The dominant *Elytropappus rhinocerotis* is tolerant of both the heavier soils but is also flammable and fire dependent. The species has characteristics that combine the climatic, edaphic and disturbance preferences of both Fynbos and Succulent Karoo species. It is possible, therefore, that Renosterveld exists as a flammable "skirt" that separates the two biomes, and further limits Succulent Karoo elements proper from establishing at higher elevations, despite the extension of loam soils upslope. Marginal habitats on the northern edges of the Fynbos Biome where Succulent Karoo and Fynbos are not separated by Renosterveld are at the greatest risk of being replaced by Succulent Karoo species in the event of a severe post-fire drought that could slow or prevent regeneration of Fynbos vegetation, and should therefore be considered important monitoring sites for early signs of a biome shift. More commonly however, drought tolerant Renosterveld may provide a resilient flammable interface on heavier shale soils that would prevent the invasion of Fynbos vegetation by Succulent Karoo elements.

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